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Preliminary

A Possible Role of Oxalate Produced in the Symbiotic Culture System with a Host Plant *Pinus densiflora* and a Mycorrhizal Fungus *Lactarius hatsudake*[*1]

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Introduction

Mycorrhizal fungi in mycorrhizae of terrestrial plants are believed to play an important role in taking up water and nutrients such as nitrogen, phosphorus, minerals from soil[1]. As a possible mechanism facilitating phosphorus uptake by the host plant, oxalate secreted by ectomycorrhizal fungi has been proposed to liberate phosphate from fixed phosphate in calcium and aluminum precipitates[2,3]. However, the growth promotion of woody plants by the addition of oxalate has not been reported. Furthermore, few reports have been published on the production of organic acids in an axenic symbiotic culture of the host plant with the ectomycorrhizal fungi[4,5].

We have established the symbiotic system of *Pinus densiflora* associated with *Lactarius hatsudake* in vermiculite culture. A possible role of oxalate is discussed in relation to symbiotic interactions between *P. densiflora* and *L. hatsudake*.

Materials and Methods

Pinus densiflora seedlings were transplanted into the culture of *L. hatsudake* in vermiculite culture system containing glucose (1%) and yeast extract (0.1%), and was grown at 21°C under 16 hr-light (3,000 lx) and 8 hr-dark conditions. The organic acids extracted according to the modified method of Yoshida *et al.*[6] were analyzed by GC-MS. The effect of oxalate on the growth of *P. densiflora* was investigated.

Results and Discussion

Ectomycorrhizae were synthesized on the roots of *P. densiflora* associated with *L. hatsudake* during cultivation of 82 days. As a result, a thick mantle and a well-developed epidermal Hartig net were observed.

Compared with non-inoculated seedlings as a control, seedlings inoculated with *L. hatsudake* showed significant increase in total dry weight (150%), seminal root length (150%) and the number of lateral roots longer than 10 mm (160%) (Table 1). The extent of the growth enhancement recognized in the present investigation was almost similar to that previously reported for Sitka spruce and Japanese Larch inoculated with *Paxillus involutus* or *Suillus grevillei*[7], and for *Pinus wallichiana* and *P. densiflora* inoculated with *Suillus granulatus*[8].

The amounts of organic acids accumulated in the three different cultures, i.e. free-living fungal culture, symbiotic culture of *P. densiflora* associated with *L. hatsudake*, and non-inoculated control *P. densiflora* culture were compared. Lactate, oxalate, succinate, malate and citrate were identified in the symbiotic culture. Oxalate accumulated as the major organic acid in both free-living fungal culture and symbiotic culture. However, there was no statistically significant difference in the amounts of oxalate

Table 1. Changes in root length and dry weight of *Pinus denisiflora* associated with and without (control) *L. hatsudake* during cultivation.

Culture days	Conditions	Length (mm)		Number of lateral root longer than 10 mm	Dry weight (mg)
		Shoot	Seminal		
82	Control	29.2±5.8	147.7±15.7	4.5±1.7	28.6±5.1
	Symbiotic	31.9±6.5[NS]	221.4±45.2*	7.4±2.2*	43.6±3.3**

The significance of differences between means of treatments and controls (n=5) was tested by Student's *t*-test. The author repeated the experiment three times. Similar reculs were always observed. *: $P<0.05$; **: $P<0.01$; [NS]: not significance.

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accumulated between free-living and symbiotic cultures. Thus, it is necessary to compare the amounts of oxalate accumulated near the ectomycorrhizae and the other parts in the same vermiculite culture in order to elucidate the effect of ectomycorrhizal symbiosis on the production of organic acids. However, more than 100 times the amount of oxalate accumulated in symbiotic culture compared to the non-inoculated *P. densiflora* culture. Furthermore, the seminal root length and dry weight of *P. densiflora* were enhanced by 160% and 150%, respectively, by the addition of oxalate (0.3 mM) whose concentration was almost the same as that determined in the symbiotic culture. Thus, the results suggest that oxalate produced by the fungi was involved in the growth promotion of *P. densiflora* in the late stage during symbiotic cultivation.

Further research is needed to elucidate the mechanism for oxalate biosynthesis and the role of organic acid in the symbiosis between woody plants and ectomycorrhizal fungi.

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